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## Revitalizing the drug pipeline

Farrell, Luchi; Lo, R.; Wanford, J. J.; Jenkins, A.; Maxwell, A.; Piddock, Laura

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## Revitalising the drug pipeline:

## AntibioticDB, an open access database to aid antibacterial research and development

L.J. Farrell<sup>1#</sup>, R. Lo<sup>2,3#</sup>, J.J. Wanford<sup>1#</sup>, A. Jenkins<sup>4</sup>, A. Maxwell<sup>3</sup> and L.J.V. Piddock<sup>1\*</sup>

<sup>1</sup> Institute of Microbiology & Infection, College of Medical & Dental Sciences, University of Birmingham, Edgbaston, Birmingham, UK. B15 2TT

<sup>2</sup> School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, UK.  
NR4 7TJ

<sup>3</sup> Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, UK.  
NR4 7UH

<sup>4</sup> British Society for Antimicrobial Chemotherapy, Griffin House, 53 Regent Place, Birmingham, UK.  
B1 3NJ

<sup>#</sup>*Authors are equal first author*

\*Corresponding author:

email: [l.j.v.piddock@bham.ac.uk](mailto:l.j.v.piddock@bham.ac.uk)

Telephone:

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Running title: Discovery of new antibiotics

23 The current state of antibiotic discovery, research and development is insufficient to respond to the  
24 need for new treatments for drug-resistant bacterial infections. The process has changed over the  
25 last decade with most new agents in phases 1-3, or recently approved, having been discovered in  
26 small and medium-sized enterprises (companies; SMEs) or academia. These have then been  
27 licensed or sold to large companies for development with the end goal of taking them to market.  
28 However, early drug discovery and development, including the possibility of developing previously  
29 discontinued agents would benefit from a database of antibacterial compounds, to be scrutinised  
30 by the developer. This article describes the first free, open-access searchable database of  
31 antibacterial compounds, including discontinued agents, drugs under pre-clinical development and  
32 those in clinical trials: AntibioticDB ([AntibioticDB.com](https://AntibioticDB.com)). Data were obtained from publicly  
33 available sources. This article summarises the compounds and drugs in AntibioticDB including  
34 their drug class, mode of action, development status and propensity to select drug-resistant  
35 bacteria. AntibioticDB includes compounds currently in pre-clinical development and 834 that have  
36 been discontinued and that reached varying stages of development. These may serve as starting  
37 points for future research and development.

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39

## Introduction

In 2009, the WHO declared antibiotic resistance one of the biggest threats to mankind.<sup>1</sup> One answer to the crisis seems simple: to generate new antibiotics. However, it takes approximately 10-15 years from the discovery of a compound, to progress through pre-clinical and clinical development before a medicine can be licensed and then marketed.<sup>2</sup> Furthermore, the average expenditure required to research and develop a compound is estimated at ~\$350 million<sup>3</sup> (not including failures which can increase the cost to ~\$5 billion)<sup>4</sup>. This cost could bankrupt a small or mid-size company. Once an antibiotic has been discovered, data must be provided to show a safety profile suitable for human testing. This pre-clinical development phase typically provides animal pharmacokinetic data, toxicity profiles and efficacy against the bacterial target.

Before compounds can be tested in human clinical trials, an institution must apply to the appropriate national or regional drug regulatory authority indicating that, based upon pre-clinical data, the drug is deemed safe to be tested in humans. In the USA, an Investigational New Drug (IND) application from the United States FDA must be granted before testing can commence. A similar process exists for the EU via the EMA, and in Japan and China. Typically, 17.3% of antimicrobial compounds in pre-clinical development proceed to phase 1 clinical trials.<sup>2</sup> Once an IND application has been granted, pre-marketing clinical trials are split into three phases (Table 1).

There is no doubt that the antibiotic pipeline needs revitalisation; however, the answer may not only be the development of new drugs, but also re-investigating compounds previously discontinued. Unfortunately, no database exists that collectively records the discovery of compounds and those in pre-clinical and clinical development with those that did not become approved drugs, or the reasons for the lack of development or approval. There is an existing database of microbial compounds, but this only provides chemical and physical data on some drugs.<sup>5</sup>

This article describes the first publicly accessible free database of antibacterial compounds, AntibioticDB. This includes links to data on discovery, research and clinical trials, those awaiting approval from the FDA/EMA and discontinued compounds. AntibioticDB aims to serve as a platform for future research, antibiotic discovery and development.



## Sources of antibacterial compounds

Compounds and drugs were identified by reading material from numerous sources including from (1) the ASM Interscience Conference of Antimicrobial Agents and Chemotherapy (ICAAC) or European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) conferences from 1961 to 2016; (2) Journal of Antimicrobial Chemotherapy, Antimicrobial Agents and Chemotherapy, Journal of Medicinal Chemistry, and Bioorganic and Medicinal Chemistry Letters; (3) Google patent searches (<https://patents.google.com>) with the search terms 'antibiotic' and 'antibacterial'. Additional names of compounds and drugs were obtained by discussion with key opinion leaders who had worked in the Pharmaceutical industry. Once names had been obtained, information on each compound/drug was obtained by additional internet searches e.g. on PubMed. Where available, links to published abstracts are included in AntibioticDB. For abstracts before 2013, the year the compound was first described is indicated. Wherever possible, any compound described in pharmaceutical or biotech company literature or website and/or by research institutes and/or universities has also been included. If a compound has been developed by an organisation with a website that details the current status of the compound, this website is listed. If a compound has been patented, the web address is indicated regardless of whether the patent has expired or not. Information regarding drug patents was primarily obtained by Google's patent search feature. Drugs in clinical trials included in AntibioticDB were not limited to those only under FDA or EMA approval, but also included outside drug monitoring organisations such as, but not limited to, the Pharmaceuticals and Medical Devices Agency, Japan (PMDA). Table 2 shows the definitions of terms used in AntibioticDB. Bracketed compounds in AntibioticDB represent the most promising compounds of a series of analogues. Information on the inferior derivative compounds can often be found in the reference provided.

## Limitations of AntibioticDB

The current focus of AntibioticDB is compounds active against Gram-positive and/or negative bacteria; compounds that target *Mycobacterium tuberculosis* are not currently included. It is intended that AntibioticDB will be continually updated and extended. Individuals and organisations

97 are invited to contribute information subject to peer review by BSAC. AntibioticDB has a short  
98 web-based form to facilitate this process.

99 In contrast to the abstracts presented after 1990, in the earlier years of ICAAC, very few of the  
100 presented abstracts were uploaded in full to the internet, meaning that available data on some of  
101 the compounds is very scarce. In this case, AntibioticDB serves as a platform for interested  
102 parties to contact original authors to obtain information directly that cannot be found elsewhere in  
103 the literature.

104

## Results

AntibioticDB, comprises two types of data. Firstly, antibacterial compounds in current development and for which data has been published since April 2013. This information is indicative of the current antibiotic development pipeline. Secondly, compounds described between 1961 and 31st March 2017 that were discontinued at varying stages of development. There are 147 pre-clinical compounds listed currently under research and/or development; 77 have some activity against Gram-negative bacteria. Only six of these compounds state they specifically target MDR Gram-negative infections; examples include NAB-739, a polymyxin derivative (Northern Antibiotics) and FSI-1686 a carbapenem (Merck & Co.). Some companies have focused on creating analogues of pre-existing compounds with a better pharmacology profile than predecessor compounds of the same class. As a result, some novel compounds are being developed that belong to pre-existing drug classes. Kibdelomycin, a type II topoisomerase inhibitor (Merck & Co), targets *C. difficile* infections and has a low propensity to select resistant bacteria.<sup>6</sup> Nabriva Therapeutics has an on-going pre-clinical programme investigating extended spectrum pleuromutilins (ESPs), specifically aimed at Gram-negative bacteria.<sup>7</sup>

Data from AntibioticDB demonstrates that as of 31 March 2017, there were 53 compounds in active research and development: 12 in phase 1, 20 in phase 2, 19 in phase 3, and 2 in pre-registration. A few compounds in clinical trials are claimed to display novelty in their field with unique modes of action that currently display no modes of resistance. For instance, brilacidin is a member of a new class modelled on host defence proteins (HDP-mimetics); (defensin mimetics). Two antimicrobial peptides are in clinical trials: LTX-109 (Lytixar<sup>™</sup>, Lytix Biopharma) and Pexiganan<sup>™</sup> (Dipexium Pharmaceuticals); both are topical agents for Gram-positive infections. Radezolid (Melinta Therapeutics, USA) is an oxazolidone currently in phase 2 that has shown successful results against uncomplicated skin and skin-structure infections (uSSS) and community-acquired pneumonia (CAP). There are currently 834 discontinued compounds in AntibioticDB, some of which are available to purchase for research.



## 132 **Compounds in AntibioticDB**

### 133 *Aminoglycosides (20 in AntibioticDB)*

134 Aminoglycosides are broad-spectrum agents derived from Streptomyces natural products and  
135 contain amino sugar subgroups. They act via inhibition of protein synthesis through binding to the  
136 ribosomal 30S subunit. There are currently two listed in AntibioticDB currently in research and  
137 development, one in pre-clinical and one in phase 3 trials. FY-901, currently in pre-clinical  
138 development by Changzhou Fangyuan Pharmaceutical, China, is being developed to treat MRSA.  
139 Achaogen, USA, has developed plazomicin (ACHN-490) an aminoglycoside with Gram-negative  
140 activity including multidrug-resistant Enterobacteriaceae. Following results from two phase 3  
141 studies, EPIC (urinary tract infections (UTIs)) and CARE (bacteraemia), Achaogen expect to  
142 submit a New Drug Application in the USA and a Marketing Authorization Application (MAA) in  
143 Europe during 2017/8.<sup>8</sup> The development of 18 aminoglycosides was discontinued, many due to  
144 intolerable levels of toxicity. One example is TS2037 (Meiji Seika Pharma., Japan), a derivative of  
145 arbekacin, which showed good *in vitro* broad-spectrum activity, but was discontinued because of  
146 its high levels of nephrotoxicity.

### 147 *Anthracyclines (Three in AntibioticDB)*

148 Anthracycline IT-62-B was reported by Taisho Pharmaceuticals (Japan) as having Gram-positive  
149 activity. Consistent with the current use of anthracyclines as anti-tumour chemotherapeutics,<sup>9</sup> this  
150 molecule was discontinued as an antibacterial due to its cytotoxicity in humans.

### 151 *Antibodies (Eleven in AntibioticDB)*

152 Antibodies are immune effector molecules that identify and begin the cascade leading to  
153 eradication of foreign material (including bacteria). This is achieved by opsonisation of the target  
154 bacteria by cells of the immune system. Attenuation of pathogenic bacteria can be by directly  
155 blocking a bacterial component essential to virulence (e.g. adhesins or toxins) through binding of  
156 specific monoclonal antibodies. These differ from the mechanisms of action of typical antibiotics,  
157 suggesting that cross resistance is unlikely, making monoclonal antibody therapy an attractive  
158 option for treatment of bacterial disease.<sup>10</sup> An example of an indirectly neutralising antibody in

159 AntibioticDB is Thravixa (Emergent Biosolutions, USA), which targets the *Bacillus anthracis* toxin,  
160 reducing the ability of the bacterium to cause disease.<sup>11</sup> Studies in rabbits, and phase 1 clinical  
161 trials indicate that the antibody is well-tolerated and decreases mortality rates in the animal model.  
162 Further identification of novel, highly-conserved bacterial targets for antibody therapy is required to  
163 render these technologies a viable therapeutic option.

#### 164 *Antimicrobial peptides (AMPs) (60 in AntibioticDB)*

165 AMPs are naturally-occurring peptides, often present in the innate immune system, that  
166 demonstrate antibacterial activity, and are evolutionarily conserved with a diverse range of  
167 functions. They are not only effective as antibiotics but also demonstrate activity against fungi and  
168 viruses.<sup>12</sup> Most AMPs act against bacteria via membrane permeabilisation, which is possible due  
169 to the AMP's amphipathic structure, allowing them to bind to, and penetrate, bacterial membranes.  
170 Unfortunately, most AMPs have toxicity issues, hampering their development into therapeutic  
171 drugs.<sup>13</sup> One of the main problems with AMPs derived from human immune effectors is the risk  
172 that bacteria may become resistant thus making the immune system redundant. Creating semi-  
173 synthetic AMPs using prediction models can help reduce toxicity and improve efficacy<sup>14</sup> as well as  
174 generate variation in the AMP structure. However, due to poor pharmacokinetics of many AMPs,  
175 they have been limited to exploration as topical applications.<sup>15</sup> Of the 60 listed, research into 23 is  
176 no longer active. Thirty-seven products are in active pre-clinical development including agents  
177 with activity against both Gram-negative and Gram-positive bacteria. AA-139 (Arenicin),  
178 developed by Adenium Biotech, Denmark, is currently in pre-clinical development and shows  
179 activity against a variety of Gram-negative bacteria including *Escherichia coli*, *K. pneumoniae*, *P.*  
180 *aeruginosa* and *A. baumannii*. NAI-603, developed by NAICONs, Italy and NAI-107 developed by  
181 Sentinella Pharmaceuticals, Inc. (previously owned by NAICONs), are both currently in pre-clinical  
182 development for MRSA. Two AMPs, Lytixar (LTX-109 by Lytix Biopharma, Norway) and  
183 Pexiganan by Dipexium Pharmaceuticals (DPRX) are currently in phase 2 and 3, respectively, for  
184 topical administration.

185 Defensins are a cationic subgroup of AMPs which play a crucial role in innate bacterial immunity.<sup>16</sup>  
186 Due to the defensin's cationic (positive) charge they can bind to negatively-charged bacterial

187 membranes, producing pore-like structures and enhancing permeability. Brillacidin (Cellceutix,  
188 USA), a defensin-mimetic compound is currently in clinical trials for a wide-range of non-infective  
189 clinical indications including ulcerative colitis and mucositis in addition to phase 2 clinical trials of  
190 acute bacterial skin and skin structure infections (ABSSSI), results of which demonstrated non-  
191 inferiority to daptomycin.<sup>17</sup> Celluceutix is investigating Brillacidin and similar compounds pre-  
192 clinically for the management of Gram-negative and fungal infections.

193 Bacteriocins are AMPs produced by bacteria to defend against competing prokaryotes. Academia  
194 has primarily focused on the lantibiotics, which facilitate their action by inhibiting cell wall  
195 biosynthesis leading to membrane instability and cell death.<sup>18</sup> Therapeutic use has been  
196 hampered by their narrow spectrum, expense of production and limited tissue distribution.  
197 AntibioticDB contains examples of several compounds that could serve as a scaffold for  
198 optimisation and development into therapeutic drugs. For example, Asahikasei Pharma Corp.,  
199 Japan was developing API7444 for the treatment of MRSA and penicillin-resistant *Streptococcus*  
200 *pneumoniae*. While this compound showed potent activity *in vitro*, its activity against MRSA in  
201 mouse models was markedly lower than that of existing treatments and so the compound was  
202 discontinued in 2004.<sup>19</sup>

#### 203 *Bacteriophage endolysins (15 plus four bacteriophage delivery systems in AntibioticDB)*

204 Endolysins (or lysins) are hydrolytic enzymes produced by bacteriophages that target the  
205 peptidoglycan layer of bacteria triggering lysis. Several potential candidate compounds such as  
206 CF-301 has completed phase 1 (ContraFect, USA). This compound is under development for the  
207 treatment of resistant *S. aureus* bloodstream infections. Phico Therapeutics have developed a  
208 novel bacteriophage engineered to deliver a DNA-binding protein with cidal antibacterial action.  
209 Their first product to enter clinical trials is SASPject™ PT1.2, studied in the treatment of *S. aureus*-  
210 related infections. Phico have further products in pre-clinical studies for the treatment and  
211 management of *Pseudomonas*, *Klebsiella* and *E. coli* infections. Fifteen further compounds are  
212 listed in pre-clinical development with activity mainly against Gram-positive bacteria. The only  
213 exception is PlyF307 (Laboratory of Bacterial Pathogenesis and Immunology, The Rockefeller

214 University, New York, New York, USA) that demonstrated activity against *A. baumannii* biofilms  
215 both *in vitro* and *in vivo*.<sup>20</sup>

216 Endolysins are ineffective against Gram-negative bacteria since their outer membrane prevents  
217 access to the peptidoglycan wall.<sup>21</sup> A novel approach to circumvent this problem is to combine  
218 endolysins with an antimicrobial peptide in order to breach the outer membrane. One example is  
219 Art-175 (Laboratory of Gene Technology, KU Leuven, Belgium), which combines an endolysin with  
220 a targeting peptide that transports the endolysin through the outer membrane of Gram-negative  
221 bacteria. Art-175 demonstrated potent activity against *P. aeruginosa*,<sup>22</sup> suggesting potential in the  
222 development of future therapies.

223 In addition to the use of therapeutic phage lysins, whole bacteriophage therapy has long been  
224 considered a potential treatment for antimicrobial-resistant infections. While this has gained  
225 traction in some parts of the world, further development is required, especially with the potential for  
226 bacteria to develop phage resistance.<sup>23</sup>

#### 227 *Beta-lactam antibiotics (220 in AntibioticDB)*

228 Beta-lactams are a broad class, all containing the characteristic four-membered lactam ring, and  
229 include: carbapenems, cephalosporins, monobactams and penicillins. Their action is facilitated  
230 through interaction with penicillin-binding proteins (PBPs), enzymes involved in peptidoglycan and  
231 cell wall biosynthesis, causing cell lysis through weakening of the peptidoglycan layer. The  
232 foremost resistance determinant for this class of drugs is deactivation by bacterial beta-  
233 lactamases.

234 There are 47 carbapenems listed; four are under active investigation, two are in pre-clinical studies  
235 and two are in clinical trials. In pre-clinical investigations FSI-1671 and FSI-1686 (Achillon/FOB  
236 Synthesis) have demonstrated efficacy against Gram-negative bacterial infections.<sup>24</sup> Imipenem  
237 with relebactam (MK-7655) (Merck, USA) is a carbapenem/beta-lactamase inhibitor combination  
238 that is currently moving into phase 3 clinical trials for the management of hospital-acquired and  
239 ventilator-acquired pneumonia and against imipenem resistant infections. In 2016, a phase 3  
240 study of the meropenem-vaborbactam (another carbapenem-beta-lactamase inhibitor) combination

241 product (Medicines Company, USA) in complicated UTIs was carried out. In February 2017, a New  
242 Drug Application (NDA) was filed with the FDA.

243 Of the 98 cephalosporins listed in AntibioticDB only four remain in active development.  
244 Cephalosporins are semi-synthetic agents based on the natural product produced by  
245 *Cephalosporium acremonium*. Two of the four cephalosporins listed are currently in pre-clinical  
246 development. The fate of one of these products is however unsure; CB-027 a cephalosporin was  
247 in the Cubist Pharmaceuticals programme and had demonstrated broad-spectrum activity against  
248 several drug-resistant strains including MRSA, *P. aeruginosa* and *K. pneumoniae*.<sup>25</sup> Following the  
249 takeover of Cubist by Merck no evidence can be found that development of this product is  
250 continuing. Two further cephalosporin-containing products cefiderocol (a siderophore  
251 cephalosporin) and the combination ceftaroline/avibactam are currently in phase 3. Currently there  
252 is only one licensed monobactam in clinical use, aztreonam. A combination product of  
253 aztreonam/avibactam is currently in active development in phase 2.

254 Two hundred and three beta-lactams (including combination products) have been identified which  
255 are no longer the focus of active research and development. These include 43 carbapenems, 98  
256 cephalosporins, 15 monobactams and 14 penicillins.

#### 257 *Efflux inhibitors (13 in AntibioticDB)*

258 Efflux pumps are trans-membrane proteins that can extrude diverse substrates, and constitute one  
259 of the most common mechanisms underlying intrinsic drug resistance;<sup>26</sup> inhibiting their activity  
260 increases bacterial susceptibility to the compounds extruded. MBX 2319 (Microbiotix Inc, USA) a  
261 pyranopyridine compound in pre-clinical studies inhibits the action of the *E. coli* AcrB multi-drug  
262 resistance efflux pump. AcrB can extrude a wide array of antibiotics including chloramphenicol,  
263 fluoroquinolones and beta-lactams. Use of inhibitors should restore activity to these drugs.  
264 Optimisation of the molecular scaffolds of these inhibitors for enhanced efficacy and species  
265 specificity is under active research.

266 Development has stopped for the eleven remaining compounds. One, MC-04124, was only  
267 discontinued following the closure of Essential Therapeutics, who were developing several efflux  
268 inhibitors. Molecules such as these may make good candidates for continued development.

269 *Fab inhibitors (Twelve in AntibioticDB)*

270 Bacterial fatty acid synthesis (FAS-II) is maintained by a series of mono-functional enzymes that  
271 make up the FAS-II pathway. FabI or Enoyl-ACP reductase, is a key enzyme in the final steps  
272 and is conserved throughout most bacterial species. There are eight FabI inhibitors undergoing  
273 investigation, with four currently in clinical trials.<sup>27-29</sup> Debio1450, Debio1452 (Group, Switzerland)  
274 and CG-400549 (CrystalGenomics, South Korea) are currently in phase two and being developed  
275 for the treatment of acute bacterial skin and skin structure infections. FAB001/MUT056399 (Fab  
276 Pharmaceuticals, India) is a narrow-spectrum FabI inhibitor being developed against MRSA.<sup>27</sup> The  
277 four FabI inhibitors currently in pre-clinical development are mostly aimed at Gram-positive  
278 bacterial infections with the exception of PT52 and PT68 (Diphenyl ethers) (Department of  
279 Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, Colorado, USA),  
280 which has demonstrated activity against the Gram-negative bacteria *Burkholderia pseudomallei*.<sup>30</sup>

281 *FtsZ inhibitors/cell division inhibitors (Two in AntibioticDB)*

282 FtsZ is the earliest protein involved in bacterial cytokinesis, its closest homologue in eukaryotic  
283 cells is tubulin; there is interest in utilising FtsZ and other crucial proteins in the bacterial cell  
284 division pathway as potential targets.<sup>31</sup> AntibioticDB contains two FtsZ inhibitors, TXA-709 and  
285 PC190723, both in pre-clinical development with Taxis Pharmaceuticals incorporated.

286 *Glycopeptides (including lipoglycopeptides) (29 in AntibioticDB)*

287 Glycopeptides are glycosylated non-ribosomal peptides, comprising both natural and semi-  
288 synthetic products, showing activity against Gram-positive bacteria. They bind to a fragment of the  
289 outer peptidoglycan layer, D-alanyl-D-alanine, causing inhibition of transglycosylation and  
290 transpeptidation, disrupting cell wall synthesis and leading to cell lysis and death. Due to poor  
291 permeability and active efflux, glycopeptides have limited activity against Gram-negative bacteria.  
292 We include lipoglycopeptides, which are semisynthetic compounds produced through the addition  
293 of a lipophilic side chain to the glycopeptide base. Currently there is one agent in pre-clinical  
294 development, three in clinical development. In addition, telavancin, dalbavancin and oritavancin  
295 have recently received marketing approval. Ramoplanin (Nanotherapeutics, USA) and TD-1607  
296 are currently in phase two and one, respectively. TD-1607, a glycopeptide-cephalosporin hybrid, is

297 being tested in the management of acute bacterial skin and skin structure infections and  
298 ramoplanin is being tested for the treatment of *Clostridium difficile*.<sup>32</sup>

299 Development of 23 glycopeptides in AntibioticDB has been discontinued. Following the takeover  
300 of Wyeth by Pfizer AC98-6556, their 'cell wall synthesis inhibitor' research programme was  
301 discontinued in 2009.

#### 302 *Lincosamides (three in AntibioticDB)*

303 Lincosamides inhibit protein synthesis by affecting the assembly of the 30S ribosomal complex.<sup>33</sup>  
304 AntibioticDB contains three lincomycin analogues and derivatives, with very little academic pursuit  
305 beyond discovery. Most of these compounds had adverse toxicological effects in humans,  
306 although these were not directly cited as the reason for discontinuing research. Examples include  
307 rancomycin 1 and 2,<sup>34</sup> which showed potent broad-spectrum activity, but were discontinued at the  
308 preclinical stage due to toxicity.

#### 309 *Lipopeptides (Seven in AntibioticDB)*

310 With discovery of daptomycin (1986), lipopeptides represent the latest antibiotic class to be  
311 approved. They consist of linear or cyclic peptides with a fatty acid group covalently linked to the  
312 N-terminus. It is thought that they bind to the bacterial cytoplasmic membrane and aggregate.  
313 One lipopeptide, surotomycin (Cubist), was in phase 3, testing its use against *C. difficile*-  
314 associated diarrhoea; however, the programme stopped following acquisition of Cubist by Merck.

#### 315 *LptD/Imp inhibitor (one in AntibioticDB)*

316 Murepavadin (POL7080 Polyphor, Switzerland) is currently in phase two. It is a protein epitope  
317 mimetic LptD inhibitor being developed for the treatment of *P. aeruginosa* ventilator-associated  
318 bacterial pneumonia, lower respiratory tract infections and bronchiectasis.<sup>35</sup>

#### 319 *Macrolides and Macrocycles (59 in AntibioticDB)*

320 These agents are based on naturally-occurring polyketides, produced by bacteria such as  
321 *Micromonospora*.<sup>36</sup> The macrolide ring gives the compounds their antibacterial functionality and  
322 allows reversible binding to the 50s ribosomal subunit. Three macrolides are currently under  
323 active investigation. Solithromycin (Cempra Inc.) has completed phase three for community-

324 acquired pneumonia,<sup>37</sup> however its 'new drug application' to the Food and Drugs Administration  
325 (FDA) in the US was rejected in December 2016. Before a re-application can be made the FDA  
326 requested further clinical safety information and assurances on the manufacturing facility. A  
327 second macrolide, nafithromycin (Wockhardt) will shortly be entering phase 2 for the treatment and  
328 management of community-acquired pneumonia. RBx 14255 (Department of Infectious Diseases,  
329 New Drug Discovery Research, Ranbaxy Research Laboratories, R & D, Gurgaon, India) is  
330 currently in pre-clinical development for the treatment of infection by macrolide-resistant  
331 *Streptococcus pneumoniae*.

332 Fifty-six macrolides in AntibioticDB were discontinued (24 categorised as ketolides and four  
333 azolides) many due to adverse toxic effects in humans or inferior activity to similar, already  
334 marketed compounds. Several macrolides were also discontinued due to poor stability *in vivo*.  
335 One example is diffidin (Merck & Co., USA), which, despite showing broad-spectrum activity  
336 against aerobic and anaerobic bacteria, was unstable at differing pHs and easily oxidised. This  
337 drug showed little activity in a mouse model when administered subcutaneously, but was highly  
338 effective when administered through intraperitoneal injection, suggesting that metabolism  
339 prevented it from reaching the infection site.

#### 340 *Moenomycins (One in AntibioticDB)*

341 Moenomycins act through direct inhibition of peptidoglycan glycosyltransferases, which are crucial  
342 in the last stages of bacterial cell wall synthesis. Research into therapeutic use has been limited  
343 due to suboptimal pharmacokinetics, but they represent an attractive scaffold for antibiotic  
344 discovery and development. They have recently been shown to be active against multi-drug  
345 resistant *Helicobacter pylori*.<sup>38</sup>

#### 346 *Nanoparticles (Three in AntibioticDB)*

347 Nanoparticles are between 0.1 and 100 nm; their small volume to surface area ratio gives them  
348 unique properties that can be manipulated to target specific bacterial components via novel  
349 mechanisms. In 2011 IBM developed 'ninja particles', which were biodegradable nanoparticles  
350 that could target MRSA, and act through a similar mechanism as some immune effectors. These



351 ninja particles target the membrane and cause instability, resulting in lysis. There was also a low  
352 propensity to select for resistance.<sup>39</sup>

353 It is well-documented that metal alloys have antibacterial properties,<sup>40</sup> and two nanoparticles  
354 derived from heavy metals are in pre-clinical development for the treatment of infectious disease,  
355 including both silver (nano-Ag) and gold (nano-Au) nanoparticles. Nano-Ag's antimicrobial action  
356 is mediated by its binding to the bacterial cell membrane causing dissipation of proton motive force  
357 and membrane instability.<sup>41</sup> Gold nanoparticles appear to have a more diverse mechanism of  
358 action; they have been shown to inhibit the tRNA-binding ribosomal subunit and also to inhibit the  
359 action of ATP synthase, having a deleterious effect on bacterial metabolism.<sup>42</sup> Gold nanoparticles  
360 have also been shown to increase chemotaxis and have subsequent potential to be used in drug-  
361 delivery systems.<sup>43</sup> An issue with nanoparticles can be their efficacy in the presence of serum, as  
362 serum-protein interactions dissipate nanoparticle activity. Gnanadhas *et al.*<sup>44</sup> have demonstrated  
363 that by citrate-capping silver nanoparticles, their interaction with serum proteins could be reduced.  
364 This reduction also correlated with a higher cell uptake of free nanoparticles, thereby increasing  
365 efficacy and antibacterial activity.

#### 366 *Nitrofurans (two in AntibioticDB)*

367 Nitrofurantoin and furazolidone are currently the only nitrofurans licensed for therapeutic use.  
368 Recent studies have shown that nitrofurantoin may be a candidate for revival in the treatment of  
369 ESBL-producing *E. coli* lower UTIs,<sup>45</sup> implying that nitrofurans may be a good base for future  
370 redevelopment for treatment of susceptible organisms resistant to first line antibiotics. One  
371 example from AntibioticDB is AS17665, which was discontinued by Abbott Laboratories in 1962.  
372 While the exact reason for discontinuation is unclear, the antibiotic was shown to be primarily  
373 active against tumours, and hence toxicity is a possible concern. Despite this, the compound was  
374 shown to be active against *S. aureus*, *Streptococcus pyogenes*, *E. coli* and *Salmonella*  
375 Typhimurium.

#### 376 *Oxazolidinones (80 in AntibioticDB)*

377 Oxazolidinones (e.g. linezolid) display antibacterial activity against Gram-positive bacteria, but  
378 have poor anti-Gram-negative efficacy. Their mechanism is via inhibition of protein synthesis

379 through binding to the P-site of the ribosomal 50S subunit. Currently, four out of the six active  
380 oxazolidone compounds listed in the AntibioticDB are in clinical development. MRX-II (MicuRx  
381 Pharmaceuticals, USA) is one of two listed oxazolidinones in pre-clinical development,<sup>46, 47</sup>  
382 however any information concerning its development has not been updated since 2012, possibly  
383 indicating that it has been dropped for further research. Three oxazolidones currently in clinical  
384 trials including MRX-I (MicuRx Pharmacueticals, USA) in phase 3 studies for the treatment of skin  
385 and soft tissue infections. The remaining two compounds, radezolid and LCB01 0371, are in  
386 phase 2. Radezolid (Melinta Therapeutics, USA) has demonstrated activity against Gram-negative  
387 infections and only for *Haemophilus influenzae*.<sup>48</sup> Whilst LCB01 0371 (LegoChem Biosciences,  
388 South Korea) is under investigation for the treatment of Gram-positive infections.

389 Development of 74 oxazolidinones in AntibioticDB has ceased. One, PNU100592 (Pharmacia  
390 Corp., USA) was being developed for the treatment of MRSA. This molecule was discontinued  
391 due to its inferior activity when compared with linezolid. Many other oxazolidinones were  
392 discontinued due to high levels of toxicity. Utilisation of this molecular scaffold to reduce toxicity  
393 may represent an avenue for the development of novel protein synthesis inhibitors with reduced  
394 propensity to select for resistance. Interestingly, when combined with quinolones, the combination  
395 includes Gram-negative bacteria in the spectrum of activity.<sup>49</sup>

#### 396 *Pleuromutilins (Ten in AntibioticDB)*

397 Pleuromutilins inhibit the 50S ribosomal subunit; retapamulin, was approved for human use in  
398 2007,<sup>50</sup> but, it has been difficult to develop compounds for systemic use,<sup>50</sup> mainly due to difficult  
399 peptide chemistry. Nabriva Therapuetics, Austria, has five candidate compounds in research and  
400 development with one, lefamulin (BC 3781), progressed to phase 3. Lefamulin is under  
401 development for the treatment of acute bacterial skin and skin structure infections, community-  
402 acquired bacterial pneumonia and hospital-acquired bacterial pneumonia/ventilator-associated  
403 bacterial pneumonia.<sup>51, 52</sup>

#### 404 *Polymyxins (Two in AntibioticDB and one polymyxin analogue)*

405 Polymyxins are produced by non-ribosomal peptide synthetases in Gram-positive soil bacteria  
406 such as *Paenibacillus polymyxa*.<sup>53</sup> They have selective activity against Gram-negative bacteria,

407 targeting the lipid A component of the outer membrane.<sup>54</sup> Currently, the only polymyxin in clinical  
408 use is polymyxin E (colistin), but, due to high incidence of nephrotoxicity,<sup>55</sup> colistin is administered  
409 as an agent of last resort against infections by multi-drug resistant bacteria, such as *A. baumannii*.  
410 The two polymyxins in pre-clinical development and listed in AntibioticDB are NAB-739 and CA-  
411 824, which are being developed by Northern Antibiotics (Finland) and Cantab Anti-infectives,  
412 respectively.

413

#### 414 *Quinolones, Fluoroquinolones and Other Topoisomerase Inhibitors (146 in AntibioticDB)*

415 The targets for topoisomerase inhibitors in bacteria are DNA gyrase and DNA topoisomerase IV,  
416 which are enzymes that regulate bacterial DNA supercoiling and relaxation.<sup>56</sup> There are currently  
417 five fluoroquinolones in AntibioticDB undergoing active research and development, four are in  
418 clinical studies. Following two successful phase 3 studies, known as PROCEED, a 'new drug  
419 authorisation' for delafloxacin (Melinta Therapeutics, USA and Abbott) was submitted in October  
420 2016 for the treatment of gonococcal, skin and soft tissue infections. Zabofloxacin (Dong Wha  
421 Pharmaceuticals, South Korea) is in phase 3 for the treatment of community-acquired bacterial  
422 pneumonia and quinolone-non-susceptible gonorrhoea. Finafloxacin (MerLion Pharmaceuticals,  
423 Germany) has been licensed topically for the treatment of *otitis externa*. An oral formulation  
424 continues through phase 2 clinical studies for the management of urinary tract, intra-abdominal  
425 and, skin and soft tissue infections. Nemonoxacin (TaiGen Biotechnology Co., Taiwan) a non-  
426 fluorinated quinolone is currently in phase 3<sup>57</sup> and has promising broad-spectrum activity targeting  
427 a variety of infections including vancomycin-non-susceptible MRSA.

428 There are 114 discontinued quinolones and fluoroquinolones documented in AntibioticDB.  
429 Research and development into many of these agents was discontinued due to toxicity, although  
430 some agents were discontinued due to financial constraints. MCB 3382, a fluoroquinolone-  
431 oxalidinone hybrid, was in development by Morphachem AG before the company merged with  
432 Biovertis AG, leading to the discontinuation of their antibacterial research programme.

#### 433 *Sideromycins and sidophore antibiotics (eight in AntibioticDB)*

434 Siderophores are iron chelators, which can be exploited in a number of therapeutic processes.<sup>58</sup>  
435 Sideromycins are antibiotic moieties covalently linked to siderophores, allowing for their selective  
436 uptake into the bacterium via the native iron transport systems.<sup>59</sup> This feature is useful in the  
437 context of Gram-negative bacteria whereby the outer membrane forms a barrier to drug entry, and  
438 the concentration of some antibiotics required for entry into the cell can become toxic to humans.  
439 One such example is cefiderocol a siderophore cephalosporin, which forms a chelation complex in  
440 the presence of iron. Cefiderocol (Shionogi, Japan) has recently completed phase 3 for multi-drug  
441 resistant Gram-negative infections.

442 HKI 9924109 (Basilea Pharmaceutica AG, Switzerland) is an ampicillin adduct linked to a synthetic  
443 siderophore. This compound was discontinued at the preclinical stage due to its inability, even at  
444 high concentrations, to inhibit the growth of non-fermenting Gram-negative bacteria.<sup>60</sup> Despite the  
445 shortcomings of some siderophore-antibiotic conjugates, they remain a useful tool for targeted  
446 therapeutics.<sup>61</sup> For example, Wencewikz *et al*<sup>62</sup> have reported a siderophore-carbacephalosporin  
447 conjugate that selectively targets *A. baumannii*.

#### 448 *Streptogramins (Five in AntibioticDB)*

449 Streptogramins are produced by several *Streptomyces* species and are structurally unique, having  
450 similar mechanisms of action to macrolides and lincosamides, therefore cross resistance is  
451 possible by target modification thus limiting therapeutic use.<sup>63</sup> Examples are pristinamycin 1 and  
452 2, which are produced by *Streptomyces pristinaespiralis*. There are no streptogramins in active  
453 research and development listed in AntibioticDB.

#### 454 *Streptothricins (one in AntibioticDB)*

455 Streptothricins have broad-spectrum activity against bacteria and fungi;<sup>64</sup> their therapeutic potential  
456 has been hampered by toxicity problems, but they have been successfully utilised for the treatment  
457 of infectious diseases in crop plants; streptothricin-type antibiotics have been marketed as  
458 fungicidal agents in China.<sup>65</sup> One streptothricin compound in AntibioticDB is LL-AB 664, which  
459 was reported in 1967.<sup>66</sup> New streptothricin class antibiotics have been isolated and assessed for  
460 their antimicrobial potential recently, some of these display potent activity against a variety of  
461 bacteria, particularly *Mycobacterium tuberculosis*.<sup>67, 68</sup> If the problem of resistance to these

462 compounds and toxicity in humans can be overcome, then they may represent a new avenue for  
463 antibiotic development. There are no streptothricins in active research and development listed in  
464 AntibioticDB.

#### 465 *Sulphonamides (Two in AntibioticDB)*

466 Sulphonamides are synthetic compounds based on the industrial dye sulfachrysoidine;<sup>69</sup> they are  
467 analogues of p-aminobenzoic acid (PABA) and inhibit folic acid biosynthesis via competitive  
468 inhibition of the enzyme dihydropteroate synthetase.<sup>70, 71</sup> This competitive inhibition is  
469 bacteriostatic, disrupting DNA synthesis and bacterial growth. ABEPI 1 and 2 are currently in pre-  
470 clinical development.<sup>72</sup> Some antibiotics can act as efflux inhibitors and ABEPI 1 and 2 have been  
471 shown to inhibit the efflux activity of the nosocomial bacteria *A. baumannii*, which allows for  
472 increased accumulation of the antibiotic minocycline, and subsequent susceptibility to the drug.

#### 473 *Tetracyclines (10 in AntibioticDB)*

474 Tetracyclines are broad-spectrum cyclic antibiotics classified into two types: tetracyclines that bind  
475 to the amino-acyl-tRNA acceptor site of the mRNA ribosomal complex, causing disruption of  
476 protein synthesis, and those that demonstrate antibacterial activity via cytotoxic perturbation of the  
477 cytoplasmic membrane.<sup>73</sup> Out of the four tetracyclines in active development listed in AntibioticDB,  
478 two are in pre-clinical development, both by Tetrphase Pharmaceuticals, USA, and two are in  
479 phase 3. The two phase three compounds are omadacycline and eravacycline. Eravacycline  
480 (Tetrphase Pharmaceuticals, USA) has received mixed results from the phase 3 studies 'IGNITE'.  
481 In IGNITE1, eravacycline demonstrated non-inferiority to ertapenem in the management of  
482 complicated intra-abdominal infections. In IGNITE2, eravacycline showed inferiority to levofloxacin  
483 in the treatment of complicated UTIs.<sup>74</sup>

484 The IGNITE2 study protocol authorised intravenous to oral switch and further data analysis has  
485 suggested participants receiving solely eravacycline intravenously had a more favourable  
486 outcome. As a consequence, there is a suggestion that poor clinical outcomes were observed due  
487 to problems with the oral eravacycline formulations rather than with the parent compound; two  
488 further phase 3 studies (IGNITE3 and IGNITE4) indicated non-inferiority to meropenem in patients  
489 with polymicrobial infections. These were included in the recent NDA application.

490 Omadacycline is a semi-synthetic tetracycline being developed by Paratek Pharmaceuticals,  
491 technically classed as a novel aminomethylcycline, the first in its class.<sup>75</sup> The newer tetracyclines  
492 appear to avoid active efflux by bacteria and are effective against typical tetracycline-resistant  
493 bacteria. The mechanism underlying this phenomenon is unknown.<sup>75</sup> AntibioticDB contains four  
494 cyclic tetracyclines that have been discontinued for development as antibacterials.

#### 495 *Miscellaneous molecules (121 in AntibioticDB)*

496 A multitude of molecules that do not fit into pre-defined classes of compounds and inhibiting  
497 various bacterial functions have been reported to display antibiotic activity. The differences of the  
498 structures of these compounds and the commonly used therapeutic classes may provide avenues  
499 to identify novel molecular scaffolds, less prone to degradation by bacterial-resistance  
500 determinants. While this may not be an option in cases where resistance has been acquired by  
501 target modification, this remains a resource to bring novelty to the antibiotic research pipeline.  
502 Some agents may fit within a class but were not described as such when first reported.

#### 503 **Reasons for discontinuing compounds**

504 Few compounds in the development pipeline become drug candidates and even fewer are  
505 approved for clinical use. From analysis of the discontinued compounds, most were discontinued  
506 in pre-clinical research (Figure 1). This is as expected due in part to the large numbers of  
507 analogues that are often discarded for inferior activity. There are many factors to be considered  
508 before submission of an IND application relating to a promising drug candidate. These include: *in*  
509 *vitro* and *in vivo* activity (compounds should have non-inferior activity to other compounds and  
510 existing drugs), levels of toxicity, pharmacokinetic profile (e.g. bioavailability, half-life), cross-  
511 resistance to other antibiotics or resistance development and commercial reasons. In reality, few  
512 agents reach the stage where all these data are available, as discovery of a key impeding factor,  
513 e.g. high toxicity, may preclude any further research and development. The cited reasons for  
514 discontinuation of a particular drug given in AntibioticDB are depicted in Figure 2. As a result of  
515 the lack of further pursuit when a compound's poor potential is identified, very few reasons for  
516 discontinuation have been published.

517 Thirty-eight compounds are listed in AntibioticDB where evidence for discontinuation of research  
518 was identified as 'due to commercial reasons', company acquisition, or the 'financial circumstances  
519 of the developing company'. Examples include: the Genaera 'Magainin program' (company shut  
520 down), Essential Therapeutics (bankruptcy) and the Cubist pipeline (acquired by MSD). In these  
521 cases, the rights to company assets can be purchased. However, for many early stage  
522 compounds, there may not have been a sufficient incentive for these compounds to be purchased.  
523 Therefore, it is possible that certain compounds were overlooked, or in too early a stage of  
524 development, and that these have the potential to be taken to market without optimisation or be  
525 used as intermediates for further analysis and development.

526 In the last 15 years many large pharmaceutical companies (including Astra-Zeneca, Bristol Myers  
527 Squibb, Eli Lilly and Wyeth) discontinued antibiotic research because their pipelines had no viable  
528 compounds and/or because of economic factors. To identify novel compounds, European and  
529 American researchers and companies turned to target-based discovery to identify agents with  
530 novel modes of action, whilst Japan tended towards finding derivatives of existing, successful  
531 compounds such as fluoroquinolones and  $\beta$ -lactams. In using target-based discovery, researchers  
532 encountered many issues with entry of the antibiotics into bacterial cells. The timeline for antibiotic  
533 discovery is illustrated in Figure 3.

534 Of those agents with a documented reason for termination of development, approximately 50%  
535 were discontinued due to toxicity. Many compounds were also discarded where research revealed  
536 resistance, unfavourable pharmacokinetics or poor potency. Interestingly, following investigation  
537 beyond the published literature from the developer, some compounds were discontinued for  
538 "circumstantial reasons", suggesting that this was not due to unfavourable properties of the  
539 compound. One example of this is the compound JNJ-17155437, a ketolide antibiotic reported by  
540 Johnson and Johnson. Research and development into this compound was discontinued around  
541 about the time that questions were raised around another ketolide antibiotic being developed by  
542 the company called telethromycin (Ketek). This drug was approved by the FDA and said to be one  
543 of the first compounds of its type to circumvent antimicrobial resistance.<sup>76</sup> In the year following  
544 initial approval, several deaths were reported due to liver failure in people treated from community-

545 acquired pneumonia with telethromycin. The suboptimal safety profile of this drug and the  
546 structurally related JNJ-17155437 may have been the reason for discontinuation of the compound.



## 547 Discussion

548 AntibioticDB was assembled with the intention of generating a platform to facilitate researchers  
549 from academic or industry backgrounds to potentially research and develop previously  
550 discontinued compounds into new antibacterial drugs. Additionally, it is an accessible source of  
551 information to determine the progress of compounds currently in development. While there are  
552 databases and publications<sup>5, 77-79</sup> describing the properties of compounds and their status,  
553 including the Springer-AdisInsight and Thomson Reuter databases, these are not widely  
554 accessible and some are only available on a paid subscription basis. Furthermore, AntibioticDB  
555 includes antibacterial compounds no longer in active development, and indicates, where possible,  
556 the reasons why development was discontinued. To identify the latter, the published literature,  
557 company websites and other databases were interrogated, and individuals with extensive  
558 knowledge interviewed. It should be noted that for many of the compounds, there is little available  
559 information as they were discontinued in early pre-clinical development and there was no publicly  
560 available information.

561 Several discontinued antibiotic classes and compounds have been revisited to investigate whether  
562 there is any merit in developing them for medical use. For instance, in the 1980s daptomycin (LY  
563 146032) was under development by Eli Lilly and Co.; however, in clinical trials, muscle-skeletal  
564 damage was shown, and so development of the compound was discontinued. In 1997, Cubist  
565 Pharmaceuticals acquired the rights to this drug and after changing the dosing to IV administration  
566 once daily, daptomycin was found to be safe. It was approved by the FDA and marketed in  
567 2003.<sup>80</sup> Secondly, pleuromutilins such as tiamulin have been previously used in veterinary  
568 medicine, but there is now the prospect of developing them as therapies for humans. GSK  
569 developed retapamulin (Altabax) and currently Nabriva is developing a further three compounds,<sup>81</sup>  
570 including lefamulin, which is a candidate for treatment of community-acquired pneumonia. A third  
571 drug candidate is iclaprim. In 2009, FDA rejected Arpida's application on the grounds of  
572 incomplete data to demonstrate efficacy. In April 2015, the FDA accepted the proposal of two  
573 further phase 3 trials by Motif Bio who gained the rights to the drug and plan to continue its  
574 development.<sup>82</sup> These examples indicate that there can be merit in reinvestigating discontinued

575 antibiotics for future development, and AntibioticDB will provide a platform to facilitate this. There  
576 is a possibility that with the progression of synthetic chemistry and other areas of science, cross-  
577 disciplinary approaches may be able to optimise some old compounds to remove unfavourable  
578 characteristics and make them more useful in future.<sup>83</sup> For these agents to be developed there will  
579 need to be a financial incentive.

580 There is also the need to consider the target patient of new antibiotics. Some compounds were  
581 discontinued due to toxicity, however certain compounds e.g. colistin, despite presenting toxicity  
582 issues could progress further through the drug development pipeline. However, with the  
583 understanding of AMR, it is probable that many new antibiotics will not be widely used. Many will  
584 be kept as reserve agents for compassionate designation usage, i.e. usage in mitigating  
585 circumstances, where few other therapeutic options are available; this means that the regulatory  
586 authorities may allow companies to provide experimental drugs to people outside of clinical trials.  
587 Whilst re-investigating discontinued compounds is a possibility, there are some complications. For  
588 instance, the expertise in synthesising certain compounds or their documentation may no longer  
589 be available. This is likely for those companies that have ceased their research in this area or  
590 become bankrupt. In these cases, many derivatives may have been synthesised from a promising  
591 parental compound which demonstrated toxicity or other unfavourable properties. If the  
592 information about these derivatives has been lost, reinvestment is high risk, which highlights the  
593 importance of an open-access approach to pre-clinical and clinical development of therapeutic  
594 drugs. Another complication is that some discontinued compounds may not have been patented  
595 as a drug but as an intermediate. If the patent is still valid, another organisation synthesising  
596 derivatives, may infringe the patent. Finally, a company investigating old compounds will, to  
597 receive regulatory approval, ultimately need to be able to ensure a steady supply of drug.

598 While the cases of daptomycin and the pleuromutilins show that 'reviving' old antibiotics is possible  
599 and can be successful, it must be considered whether this is a viable path of antibiotic research  
600 and development for other discontinued compounds. It is important to note that the currently  
601 prevalent bacteria have evolved and disseminated because of selection by antibiotics in current  
602 clinical use. Therefore, further development of old and/or compounds of the same class may not

603 be productive as resistance mechanisms active against these agents may already be widely  
604 disseminated. Many of the discontinued compounds in AntibioticDB will not have been tested  
605 against current clinical isolates, suggesting a potential difference in efficacy from when they were  
606 first screened. Nonetheless, compounds that demonstrate a novel mode of action may evade  
607 current resistance issues and thus could be clinically useful. This was the case for daptomycin  
608 and the pleuromutilins.

609 The UK AMR Review has questioned the sustainability of the current R&D pipeline for antibiotic  
610 development<sup>2</sup> and indicated that for a sustainable future of antibiotic development 15 new  
611 antibacterials need to be developed every 10 years. The AMR review also noted that novelty is a  
612 direct issue for today's antibiotic development, with no new antibiotic drug classes being developed  
613 in the past 30 years (since the lipopeptide daptomycin in 1986). Currently there are 152 active  
614 pre-clinical compounds listed in the AntibioticDB. The attrition rate in drug development is well  
615 known and based on data provided by the Review on AMR on success rates it is possible that  
616 three may be approved for human use by 2025.<sup>2</sup> This demonstrates a possible gap of 12  
617 licensable compounds over 10 years. Data from the Review on AMR suggests the number of pre-  
618 clinical compounds that require testing in order to generate 15 licensed medicines is approximately  
619 590, a shortfall of 440 compounds (Figure 4).

620 Academia has an important part to play in the fight against antimicrobial-resistant infections, with  
621 the need for innovation in the field and subsequent development of antibiotics with novel  
622 mechanisms of action at its greatest. This alone however is not enough, and only with  
623 collaboration between academia, SMEs, big pharma, funding bodies, and governments can this  
624 goal be achieved. AntibioticDB, described herein provides a valuable tool for anyone involved with  
625 antibiotic discovery, research and development. By providing a history of compounds that have  
626 been discontinued with the current status of antibiotic discovery, research and development  
627 (including pre-clinical development), AntibioticDB will enable academia and industry alike to  
628 explore previously discontinued antibiotics for the treatment of the drug-resistant infections we are  
629 faced with today. AntibioticDB is an interactive database; therefore, we call upon all involved in

630    this field be it pharmaceutical companies, university groups or individuals, to help to continue to  
631    populate AntibioticDB.

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## 648 **Author contributions**

649 LJVP conceived and designed the project and wrote the manuscript. LJF reviewed source material  
650 and entered information into the database, generated Figure 4 and contributed text for the  
651 manuscript. RL reviewed source material and entered information into the database, generated  
652 Figure 3 and contributed text for the manuscript. JW reviewed source material and entered  
653 information into the database, generated Figures 1 and 2 and contributed text for the manuscript.  
654 AJ checked all entries in the database were correct as of March 2017. AM supervised RL,  
655 generated the references and contributed to the writing of the manuscript.

## 656 **Financial interests**

657 None of the authors have any competing financial interests.

## 658 **Transparency declarations**

659     None to declare.

660

## Legends to Figures

661 **Figure 1.** The stages at which antibiotic compounds in this database were discontinued for  
662 development (values from AntibioticDB 31<sup>st</sup> March 2017). As a disproportionate number of  
663 compounds are discontinued at the pre-clinical stage, the y-axis is split to reflect this. I: phase one  
664 clinical trials; II: phase two clinical trials; III: phase three clinical trials; IV: phase four clinical trials.

665

666 **Figure 2.** The reasons identified for termination of compound development (values from  
667 AntibioticDB 31<sup>st</sup> March 2017). Unknown; Toxicity, either in animals or humans; Inferior: studies  
668 showed inferiority to comparator compound. That may have been a marketed or study compound;  
669 Resistance, resistance acquired to compound within studies; Commercial, includes bankruptcy,  
670 mergers, closing R&D facilities; Clinical results, unfavourable outcomes; Pharmacokinetics,  
671 unsuitable parameters; Pharmacodynamics, unsuitable parameters.

672

673 **Figure 3.** A timeline of the discovery of the major classes of antibiotics. From 1986 to 2017,  
674 regulatory authorities have approved no new class of antibiotics; this has been termed the  
675 'discovery void'.<sup>84</sup>

676

677 **Figure 4.** Antibiotic discovery, research and developmental pipeline (values from AntibioticDB 31<sup>st</sup>  
678 March 2017). The X-axis represents the average time in years it takes to progress a compound  
679 through each clinical stage, with the final stage, Post-marketing surveillance, taking an  
680 undetermined amount of time. The percentage between each clinical stage states on average how  
681 many compounds will make it to the next stage of clinical development is based on the data  
682 provided in the Review on Antimicrobial Resistance.<sup>2</sup>

683

684 **Table 1: Phases of drug development**

Phase	Comment
1	Typically consist of a small group (20-100) studies in healthy volunteers in a controlled setting to test the compound's pharmacokinetics, toxicity and pharmacology; goal is to determine the maximum tolerated dose range that can be safely used; typically 33% of drugs in phase 1 proceed to phase 2.
2	Expand patient numbers to a few hundred; used to understand the compound's dosing requirements, efficacy and adverse effects; typically 59% continue to phase 3
3	Large, randomised, controlled, multicentre studies in which treatment by the study compound is usually compared against the currently accepted 'gold standard'; success in phase 3 is much higher than in earlier phases, typically ~76%; mostly due to the stringency of previous trials. Phase 3 provides clinical data necessary to file for a new drug application (NDA) potential marketing authorization applicant (MAA); application is usually submitted when there is sufficient data on the safety/pharmacology of the compound; typically ~80% of antimicrobial compounds awaiting approval are granted an NDA
4	Often termed as post-marketing surveillance; the compound is formulated as a medicine and been given full marketing approval; data is usually collected on the safety profile of the drug. Further research can be initiated to test the drug profile in different disease states, for combination therapies, alternative delivery systems and different subject groups if the company chooses to do so. Medicines can be discontinued or withdrawn at this stage if it becomes apparent that the medicine has intolerable adverse effects or is unsuitable for clinical practice

685  
686



<b>Term</b>	<b>Definition</b>
<b>1. Drug name</b>	Current generic name of the compound; alternate or past names are indicated in brackets
<b>2. Drug class</b>	Antibiotics can be classified in two ways: (1) by chemical structure e.g. a fluoroquinolone, and (2) based on the mechanism or target of the compound e.g. a topoisomerase inhibitor; in AntibioticDB, compounds are classified by both methods where applicable
<b>3. Development phase</b>	The highest development phase a compound has reached as of 30 March 2017, for example pre-clinical, phase 1, 2, 3, 4, or marketed
<b>4. Organisation</b>	Represents the party or individual that has been listed as the lead discoverer or developer of the compound; encompasses large pharmaceutical companies, university groups or individuals.
<b>5. Gram-negative activity</b>	Indicates a compound with activity against Gram-negative bacteria which is/was in the process of being developed to target these bacteria; includes compounds with a broad spectrum but are targeted to these bacteria during their development
<b>6. Gram-positive activity</b>	Indicates a compound with activity against Gram-positive bacteria which is/was in the process of being developed to target these bacteria
<b>7. Combination agents</b>	Indicates agents used in combination with other antibiotics or other compounds that enhance antibiotic efficacy of the compound in question
<b>8. Low propensity</b>	Criteria for selection of compounds with 'propensity to select resistant mutants' was only applied if data were available showing that bacteria had been exposed to the compound for the purpose of detecting bacterial resistance, or if resistance had been observed during clinical trial; where no data was available, this parameter was left blank in the database
<b>9. Mechanism of action</b>	The site/s of interaction of the compound with the bacterium e.g. cell wall inhibitor, DNA gyrase inhibitor
<b>10. Target bacteria</b>	If a compound has a broad spectrum of activity, comparative details of its efficacy against Gram-positive and Gram-negative organisms are provided
<b>11. Current status</b>	Gives information on the compound drug: active research (A) or inactive (I)
<b>12. Reason antibiotic not developed</b>	Indicates why a compound failed to advance further or was retracted from market. To gather this information, the Springer database 'AdisInsight' was used; Dr Lynn L. Silver, Dr Jared A. Silverman, Dr Ursula Theuretzbacher and Dr Glenn Tillotson provided additional information. Blank fields in this section indicate that the original author, or authors of subsequent pieces of work, has given no reason as to why a compound may have been dropped, which was often found to be the case for compounds predating 1990
<b>13. Citation</b>	Indicates the journal article/conference abstract that the compound was first described; provided as a web address and/or reference. If more than one source is cited brackets containing the number refers to the reference from which the information was derived. Where possible the first reference will be the first description of the drug (e.g. ICAAC abstract) and the second will be a publication that gives the broadest overview and/or most information regarding the compound

689 Bracketed compounds in AntibioticDB represent the most promising compounds discussed of a  
690 series of analogues. Information on the inferior derivative compounds can often be found in the  
691 reference provided.  
692

## References

1. Editorial. Urgently needed: new antibiotics. *Lancet* 2009; **374**: 1868.
2. O'Neill J. 2015. *Securing new drugs for future generations: The pipeline of antibiotics*. <https://amr-review.org/Publications.html>
3. Herper M. 2017. *How Much Does Pharmaceutical Innovation Cost? A Look At 100 Companies*. <http://www.forbes.com/sites/matthewherper/2013/08/11/the-cost-of-inventing-a-new-drug-98-companies-ranked/>.
4. Herper M. 2017. *The Cost Of Creating A New Drug Now \$5 Billion, Pushing Big Pharma To Change*. <http://www.forbes.com/sites/matthewherper/2013/08/11/how-the-staggering-cost-of-inventing-new-drugs-is-shaping-the-future-of-medicine/>
5. Mallocci G. 2017. *Antimicrobial compounds database*. <http://www.dsf.unica.it/~gmallocci/abdb/>
6. Miesel L., Hecht D. W., Osmolski J. R. *et al*. Kibdelomycin is a potent and selective agent against toxigenic *Clostridium difficile*. *Antimicrob Agents Chemother* 2014; **58**: 2387-92.
7. File, T., Jr., F. Bagheri, L. Bush, *et al*. A phase 2 study comparing two doses of radezolid to linezolid in adults with uncomplicated skin and skin structure infections (uSSSI). In: *Abstracts of the Forty-eighth Interscience Conference on Antimicrobial Agents and Chemotherapy - Forty-sixth Annual Meeting of the American Society of Microbiology and Infectious Diseases Society of America, Washington DC, 2008*. Abstract L-1515c. p. 126. American Society for Microbiology, Washington DC, USA
8. Archaogen. 2017. *Plazomicin: the first clinical candidate from our gram-negative antibiotic discovery engine*. <http://www.achaogen.com/plazomicin/>
9. Kawauchi T., Sasaki T., Yoshida K. *et al*. A new anthracycline antibiotic, IT-62-B, converts the morphology of ras-transformed cells back to normal: taxonomy, fermentation, isolation, structure elucidation and biological characterization. *J Antibiot (Tokyo)* 1997; **50**: 297-303.
10. Bebbington C., Yarranton G. Antibodies for the treatment of bacterial infections: current experience and future prospects. *Curr Opin Biotechnol* 2008; **19**: 613-9.
11. Malkevich N. V., Hopkins R. J., Bernton E. *et al*. Efficacy and safety of AVP-21D9, an anthrax monoclonal antibody, in animal models and humans. *Antimicrob Agents Chemother* 2014; **58**: 3618-25.
12. Izadpanah A., Gallo R. L. Antimicrobial peptides. *J Am Acad Dermatol* 2005; **52**: 381-90.
13. Dawson R. M., Liu C. Q. Cathelicidin Peptide SMAP-29: Comprehensive Review of its Properties and Potential as a Novel Class of Antibiotics. *Drug Develop Res* 2009; **70**: 481-98.
14. Wu X., Wang Z., Li X. *et al*. In vitro and in vivo activities of antimicrobial peptides developed using an amino acid-based activity prediction method. *Antimicrob Agents Chemother* 2014; **58**: 5342-9.
15. Mohamed M. F., Hamed M. I., Panitch A. *et al*. Targeting methicillin-resistant *Staphylococcus aureus* with short salt-resistant synthetic peptides. *Antimicrob Agents Chemother* 2014; **58**: 4113-22.
16. Ganz T. Defensins: antimicrobial peptides of innate immunity. *Nat Rev Immunol* 2003; **3**: 710-20.
17. Cellceutix. 2017. *Brilacidin*. <http://www.cellceutix.com/brilacidin-1/>
18. Brotz H., Sahl H. G. New insights into the mechanism of action of lantibiotics--diverse biological effects by binding to the same molecular target. *J Antimicrob Chemother* 2000; **46**: 1-6.
19. Toriya M., Shizmu K., Ishiguro M. In vitro and in vivo antimicrobial activity of a novel lantibiotic- AP17444. In: *Abstracts of the Forty-fourth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington DC, 2004*. Abstract F-1932 p. 232. American Society for Microbiology, Washington DC, USA
20. Lood R., Winer B. Y., Pelzek A. J. *et al*. Novel phage lysin capable of killing the multidrug-resistant gram-negative bacterium *Acinetobacter baumannii* in a mouse bacteremia model. *Antimicrob Agents Chemother* 2015; **59**: 1983-91.
21. Xu Z. Q., Flavin M. T., Flavin J. Combating multidrug-resistant Gram-negative bacterial infections. *Expert Opin Investig Drugs* 2014; **23**: 163-82.

- 749 **22.** Briers Y., Walmagh M., Grymonprez B. *et al.* Art-175 is a highly efficient antibacterial  
750 against multidrug-resistant strains and persists of *Pseudomonas aeruginosa*. *Antimicrob Agents*  
751 *Chemother* 2014; **58**: 3774-84.
- 752 **23.** Coward C., Grant A. J., Swift C. *et al.* Phase-variable surface structures are required for  
753 infection of *Campylobacter jejuni* by bacteriophages. *Appl Environ Microbiol* 2006; **72**: 4638-47.
- 754 **24.** Joo H., Choi W.-B., Kim D.-I. *et al.* FSI-1671, a Novel Anti-Acinetobacter Carbapenem; in  
755 vivo Efficacy against Carbapenem-Resistance Gram-Negative Bacterial Infection. In: *Abstracts of*  
756 *the Fifty-third Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO,*  
757 *2013.* Abstract F-1201 p. 117. American Society for Microbiology, Washington DC, USA
- 758 **25.** Zhang S., Mortin L.I., Li Y. *et al.* In Vivo Efficacy of CB-027 against Methicillin-Resistant  
759 *Staphylococcus aureus*, and Ceftazidime-Resistant *Pseudomonas aeruginosa* and *Klebsiella*  
760 *pneumoniae* Infections in Mice In: *Abstracts of the fifty-second Interscience Conference on*  
761 *Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2012.* Abstract F-846 p. 100.  
762 American Society for Microbiology, Washington DC, USA.
- 763 **26.** Nguyen S. T., Kwasny S. M., Ding X. *et al.* Structure-activity relationships of a novel  
764 pyranopyridine series of Gram-negative bacterial efflux pump inhibitors. *Bioorganic & medicinal*  
765 *chemistry* 2015; **23**: 2024-34.
- 766 **27.** Escaich S., Prouvensier L., Saccomani M. *et al.* The MUT056399 inhibitor of FabI is a new  
767 antistaphylococcal compound. *Antimicrob Agents Chemother* 2011; **55**: 4692-7.
- 768 **28.** Flamm R. K., Rhomberg P. R., Kaplan N. *et al.* Activity of Debio1452, a FabI inhibitor with  
769 potent activity against *Staphylococcus aureus* and coagulase-negative *Staphylococcus* spp.,  
770 including multidrug-resistant strains. *Antimicrob Agents Chemother* 2015; **59**: 2583-7.
- 771 **29.** Schiebel J., Chang A., Shah S. *et al.* Rational design of broad spectrum antibacterial  
772 activity based on a clinically relevant enoyl-acyl carrier protein (ACP) reductase inhibitor. *J Biol*  
773 *Chem* 2014; **289**: 15987-6005.
- 774 **30.** Cummings J. E., Beaupre A. J., Knudson S. E. *et al.* Substituted diphenyl ethers as a novel  
775 chemotherapeutic platform against *Burkholderia pseudomallei*. *Antimicrob Agents Chemother*  
776 2014; **58**: 1646-51.
- 777 **31.** Erickson H. P., Anderson D. E., Osawa M. FtsZ in bacterial cytokinesis: cytoskeleton and  
778 force generator all in one. *Microbiology and molecular biology reviews : MMBR* 2010; **74**: 504-28.
- 779 **32.** Kraus C. N., Lyerly M. W., Carman R. J. Ambush of *Clostridium difficile* spores by  
780 ramoplanin: activity in an in vitro model. *Antimicrob Agents Chemother* 2015; **59**: 2525-30.
- 781 **33.** Reusser F. Effect of lincomycin and clindamycin on peptide chain initiation. *Antimicrob*  
782 *Agents Chemother* 1975; **7**: 32-7.
- 783 **34.** Argoudelis A.D., Pyke T.R. Antibiotics Rancomycin I, Rancomycin II, their 2,4-  
784 dinitrophenylhydrazones, and microbiological and chemical processes for preparing the same. US  
785 Patent no. 3,476,857. Upjohn Company, Kalamazoo, MI, USA. 1969.
- 786 **35.** Demarco S.J., Zumbrunn J., Endermann R. *et al.* POL7080, a Novel Anti-*Pseudomonas*  
787 Compound: Determination of the Predictive PK/PD Parameter for Efficacy in Murine Thigh and  
788 Lung Infection. In: *Abstracts of the Forty-eighth Interscience Conference on Antimicrobial Agents*  
789 *and Chemotherapy, Washington DC, 2008.* Abstract F1-3996, p. 329. American Society for  
790 Microbiology, Washington DC, USA.
- 791 **36.** Canedo L. M., Fernandez-Puentes J. L., Baz J. P. IB-96212, a novel cytotoxic macrolide  
792 produced by a marine *Micromonospora*. II. Physico-chemical properties and structure  
793 determination. *J Antibiot (Tokyo)* 2000; **53**: 479-83.
- 794 **37.** Oldach D., Clark K., Schranz J. *et al.* Randomized, double-blind, multicenter phase 2 study  
795 comparing the efficacy and safety of oral solithromycin (CEM-101) to those of oral levofloxacin in  
796 the treatment of patients with community-acquired bacterial pneumonia. *Antimicrob Agents*  
797 *Chemother* 2013; **57**: 2526-34.
- 798 **38.** Tseng Y. Y., Liou J. M., Hsu T. L. *et al.* Development of bacterial transglycosylase inhibitors  
799 as new antibiotics: moenomycin A treatment for drug-resistant *Helicobacter pylori*. *Bioorg Med*  
800 *Chem Lett* 2014; **24**: 2412-4.
- 801 **39.** IBM. (2017) *Ninja polymers.*  
802 [http://www.research.ibm.com/featured/ninjas/index.shtml#fbid=cDH\\_mYFZXlm](http://www.research.ibm.com/featured/ninjas/index.shtml#fbid=cDH_mYFZXlm)
- 803 **40.** Hajipour M. J., Fromm K. M., Ashkarran A. A. *et al.* Antibacterial properties of  
804 nanoparticles. *Trends Biotechnol* 2012; **30**: 499-511.

805 41. Cui Y., Zhao Y., Tian Y. *et al.* The molecular mechanism of action of bactericidal gold  
806 nanoparticles on Escherichia coli. *Biomaterials* 2012; **33**: 2327-33.

807 42. Lok C. N., Ho C. M., Chen R. *et al.* Proteomic analysis of the mode of antibacterial action of  
808 silver nanoparticles. *J Proteome Res* 2006; **5**: 916-24.

809 43. Vinoj G., Pati R., Sonawane A. *et al.* In vitro cytotoxic effects of gold nanoparticles coated  
810 with functional acyl homoserine lactone lactonase protein from Bacillus licheniformis and their  
811 antibiofilm activity against Proteus species. *Antimicrob Agents Chemother* 2015; **59**: 763-71.

812 44. Gnanadhas D. P., Ben Thomas M., Thomas R. *et al.* Interaction of silver nanoparticles with  
813 serum proteins affects their antimicrobial activity in vivo. *Antimicrob Agents Chemother* 2013; **57**:  
814 4945-55.

815 45. Tasbakan M. I., Pullukcu H., Sipahi O. R. *et al.* Nitrofurantoin in the treatment of extended-  
816 spectrum beta-lactamase-producing Escherichia coli-related lower urinary tract infection. *Int J*  
817 *Antimicrob Agents* 2012; **40**: 554-6.

818 46. Gordeev M. F., Yuan Z. Y. New potent antibacterial oxazolidinone (MRX-I) with an  
819 improved class safety profile. *J Med Chem* 2014; **57**: 4487-97.

820 47. Li C. R., Zhai Q. Q., Wang X. K. *et al.* In vivo antibacterial activity of MRX-I, a new  
821 oxazolidinone. *Antimicrob Agents Chemother* 2014; **58**: 2418-21.

822 48. Lawrence L., Danese P., DeVito J. *et al.* In vitro activities of the Rx-01 oxazolidinones  
823 against hospital and community pathogens. *Antimicrob Agents Chemother* 2008; **52**: 1653-62.

824 49. Bozdogan B., Appelbaum P. C. Oxazolidinones: activity, mode of action, and mechanism of  
825 resistance. *Int J Antimicrob Agents* 2004; **23**: 113-9.

826 50. Novak R. Are pleuromutilin antibiotics finally fit for human use? *Ann N Y Acad Sci* 2011;  
827 **1241**: 71-81.

828 51. Prince W. T., Ivezic-Schoenfeld Z., Lell C. *et al.* Phase II clinical study of BC-3781, a  
829 pleuromutilin antibiotic, in treatment of patients with acute bacterial skin and skin structure  
830 infections. *Antimicrob Agents Chemother* 2013; **57**: 2087-94.

831 52. van Duijkeren E., Greko C., Pringle M. *et al.* Pleuromutilins: use in food-producing animals  
832 in the European Union, development of resistance and impact on human and animal health. *J*  
833 *Antimicrob Chemother* 2014; **69**: 2022-31.

834 53. Zavascki A. P., Goldani L. Z., Li J. *et al.* Polymyxin B for the treatment of multidrug-  
835 resistant pathogens: a critical review. *J Antimicrob Chemother* 2007; **60**: 1206-15.

836 54. Morrison D. C., Jacobs D. M. Binding of polymyxin B to the lipid A portion of bacterial  
837 lipopolysaccharides. *Immunochemistry* 1976; **13**: 813-8.

838 55. Falagas M. E., Kasiakou S. K. Toxicity of polymyxins: a systematic review of the evidence  
839 from old and recent studies. *Critical care* 2006; **10**: R27.

840 56. Bush N.G., Evans-Roberts K., Maxwell A. DNA Topoisomerases. In: Böck A, Curtiss III R,  
841 Kaper JB *et al.*, eds. *EcoSal—Escherichia coli and Salmonella: cellular and molecular biology*.  
842 Washington DC: ASM Press, 2015.

843 57. Cao G. Y., Zhang J., Zhang Y. Y. *et al.* Safety, tolerability, and pharmacokinetics of  
844 intravenous nemonoxacin in healthy chinese volunteers. *Antimicrob Agents Chemother* 2014; **58**:  
845 6116-21.

846 58. Jayasinghe S., Siriwardhana A., Karunaratne V. Natural iron sequestering agents: their  
847 roles in nature and therapeutic potential. *Int J Pharm Pharm Sci* 2015; **7**: 8-12.

848 59. Braun V., Pramanik A., Gwinner T. *et al.* Sideromycins: tools and antibiotics. *Biometals*  
849 2009; **22**: 3-13.

850 60. Shapiro S., Thenoz L., Heinisch L. *et al.* Activities of novel siderophoric beta-lactams  
851 towards Gram-negative bacteria. In: *Abstracts of the Forty-fifth Interscience Conference on*  
852 *Antimicrobial Agents and Chemotherapy, Washington, 2005*. Abstract F-156, p. 201. American  
853 Society for Microbiology, Washington DC, USA.

854 61. Ji C., Juarez-Hernandez R. E., Miller M. J. Exploiting bacterial iron acquisition: siderophore  
855 conjugates. *Future Med Chem* 2012; **4**: 297-313.

856 62. Wencewicz T. A., Miller M. J. Biscatecholate-monohydroxamate mixed ligand siderophore-  
857 carbacephalosporin conjugates are selective sideromycin antibiotics that target Acinetobacter  
858 baumannii. *J Med Chem* 2013; **56**: 4044-52.

859 63. Leclercq R., Courvalin P. Bacterial resistance to macrolide, lincosamide, and streptogramin  
860 antibiotics by target modification. *Antimicrob Agents Chemother* 1991; **35**: 1267-72.

64. Waksman S. A., Woodruff H.B. Streptothricin, a New Selective Bacteriostatic and Bactericidal Agent, Particularly Active Against Gram-Negative Bacteria. *Exp Biol Med* 1942; **49**: 207-10.
65. Changxiong Z. Zhongshengmycin, a New Agro-antibiotics. *Fine and Specialty Chemicals* 2002; **16**: 14-7.
66. Zhou Z., Xu Q., Bu Q. *et al.* Genome mining-directed activation of a silent angucycline biosynthetic gene cluster in *Streptomyces chattanoogensis*. *Chembiochem* 2015; **16**: 496-502.
67. Gan M., Zheng X., Liu Y. *et al.* Three new 12-carbamoylated streptothricins from *Streptomyces* sp. I08A 1776. *Bioorg Med Chem Lett* 2012; **22**: 6151-4.
68. Ji Z., Wang M., Zhang J. *et al.* Two new members of streptothricin class antibiotics from *Streptomyces qinlingensis* sp. nov. *J Antibiot (Tokyo)* 2007; **60**: 739-44.
69. Smith C. L., Powell K. R. Review of the sulfonamides and trimethoprim. *Pediatr Rev* 2000; **21**: 368-71.
70. Henry R. J. The Mode of Action of Sulfonamides. *Bacteriological reviews* 1943; **7**: 175-262.
71. Rollo I. M. The mode of action of sulphonamides, proguanil and pyrimethamine on *Plasmodium gallinaceum*. *Br J Pharmacol Chemother* 1955; **10**: 208-14.
72. Blanchard C., Barnett P., Perlmutter J. *et al.* Identification of *Acinetobacter baumannii* serum-associated antibiotic efflux pump inhibitors. *Antimicrob Agents Chemother* 2014; **58**: 6360-70.
73. Rasmussen B., Noller H. F., Daubresse G. *et al.* Molecular basis of tetracycline action: identification of analogs whose primary target is not the bacterial ribosome. *Antimicrob Agents Chemother* 1991; **35**: 2306-11.
74. Zhanel G. G., Cheung D., Adam H. *et al.* Review of Eravacycline, a Novel Fluorocycline Antibacterial Agent. *Drugs* 2016; **76**: 567-88.
75. Draper M. P., Weir S., Macone A. *et al.* Mechanism of action of the novel aminomethylcycline antibiotic omadacycline. *Antimicrob Agents Chemother* 2014; **58**: 1279-83.
76. Ross D. B. The FDA and the case of Ketek. *The New England journal of medicine* 2007; **356**: 1601-4.
77. Butler M. S., Blaskovich M. A., Cooper M. A. Antibiotics in the clinical pipeline in 2013. *J Antibiot (Tokyo)* 2013; **66**: 571-91.
78. de Souza Mendes Cd, de Souza Antunes A. M. Pipeline of Known Chemical Classes of Antibiotics. *Antibiotics* 2013; **2**: 500-34.
79. The Pew Trusts Charitable. 2014. *Antibiotics Currently in Clinical Development*. <http://www.pewtrusts.org/en/multimedia/data-visualizations/2014/antibiotics-currently-in-clinical-development/>
80. Eisenstein B. I., Oleson F. B., Jr., Baltz R. H. Daptomycin: from the mountain to the clinic, with essential help from Francis Tally, MD. *Clin Infect Dis* 2010; **50 Suppl 1**: S10-5.
81. Rubino C. M., Xue B., Bhavnani S. M. *et al.* Population pharmacokinetic analyses for BC-3781 using phase 2 data from patients with acute bacterial skin and skin structure infections. *Antimicrob Agents Chemother* 2015; **59**: 282-8.
82. Motifbio. 2016. *ICLAPRIM*. <http://www.motifbio.com/iclaprim/>
83. Brown D. Antibiotic resistance breakers: can repurposed drugs fill the antibiotic discovery void? *Nat Rev Drug Discov* 2015; **14**: 821-32.
84. Silver L. L. Challenges of antibacterial discovery. *Clin Microbiol Rev* 2011; **24**: 71-109.